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THE PREVALENCE OF THE VARIANTS OF THE L-FICOLIN GENE (*FCN2*) IN THE ARCTIC POPULATIONS OF EAST SIBERIA

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Abstract

L-ficolin encoded by *FCN2* gene is a crucial factor of defense against infection in humans. We studied the prevalence of the two common variants (rs17549193 and rs7851696) in aboriginal and alien populations of the Taymyr-Dolgan-Nenets Region of Krasnoyarskiy Kray, East Siberia, Russia (Nenets, Dolgans, Nganasans, Russians). We found a decreased prevalence of the rs17549193*T allele in all aboriginal populations as compared to Russians. Also, its frequency was the lowest in the Nenets among the studied populations, while frequency of the rs7851696*T allele was increased in this population. The results suggest that the Arctic populations of East Siberia are characterised by specificity of genetic make-up responsible for the activity of L-ficolin. Clinical and epidemiological studies are required to discover if these genetic features correlate with the infant infectious morbidity in East Siberian populations.

Keywords

newborns; L-ficolin; SNPs; Russia; circumpolar area; ethnic groups.

Introduction

Infant mortality in indigenous populations of the North remains very high. This situation is typical not only for Russia, but for other countries of the Arctic (USA, Canada, Norway). In recent years, research centres began paying attention on genetically determined metabolic and immune response features in children of the indigenous populations of the North which can contribute to severe course of common infectious diseases with increased mortality risk.

In the Arctic regions, respiratory tract and central nervous system infectious diseases caused by encapsulated bacteria (*Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae*) and associated with high risk of adverse outcome are most important. Common immune system defects, concerning complement system, Toll-like receptors, IgA production, are known which are associated with most severe invasive course of these diseases. Not necessarily fatal, these peculiarities of immune response can accumulate in some populations with high frequency. Associations between some genotypes and unfavourable course of several infectious diseases are known in populations of Central Asia. Other inherited defects were suggested to be associated with such the diseases course in aboriginal populations of the Arctic regions (Chapman and Hill 2012; McLaren et al. 2013; Rubicz et al. 2013; Vannberg et al. 2011).

The lectin-mediated activation of complement is one of the important pathways of the first line non-specific defence against infections. Currently, only a few molecules known to activate the lectin pathway of complement activation: the human ficolins and the collectins (mannose-binding lectin (MBL), collectin liver-1, and collectin kidney-1). All these molecules are capable to recognise surface-linked carbohydrates or acetyl groups on pathogens (Kilpatrick and Chalmers 2012; Troldborg et al. 2017). It has been supposed that individuals with combined MBL2 and ficolins deficiency may be at risk to morbidity (Bjarnadottir et al. 2016).

Ficolins are the humoral factors of innate immunity, structurally and functionally homologues to MBL. Three types of ficolins were described including L-ficolin encoded in humans by *FCN2* gene, M-ficolin (*FCN1*), and H-ficolin (*FCN3*). L-ficolin is produced in liver and circulates in the blood. Unlike MBL2, L-ficolin can additionally bind several components of cell walls of gram-positive bacteria such as *S. pneumoniae* (including encapsulated forms) and *S. aureus* (Krarup et al. 2005). Polymorphisms in promoter and exons of the ficolin genes were described which cause 20-times differences in L-ficolin concentrations in plasma, though genetic variants associated with zero plasma levels were not identified so far (Munthe-Fog et al. 2007).

The *FCN2* gene is located in 9q34.3 chromosomal region. A schematic representation of the FCN2 gene is shown in Fig. 1. A limited number of studies attempted to find links between L-ficolin concentrations, *FCN2* polymorphisms and human diseases with controversial results. Polish children with atopy and frequent respiratory infections were found to express decreased levels of L-ficolin in plasma (Cedzynski et al. 2007). No associations between recurrent infections in Dutch children and polymorphisms of the *FCN2* and *FCN3* genes were found (Ruskamp et al. 2009). Associations between *FCN2* polymorphisms and susceptibility to visceral leishmaniasis, schistosomiasis, hepatitis B, and tuberculosis were discovered (Mishra et al. 2015). However, no association between *FCN2*, *FCN3* and *MBL* haplotypes and tuberculosis was identified in Great Britain (Chalmers et al. 2015).

Taking into account that infections are the major factors of infant mortality and that ficolins are the crucial

factors of anti-infection defence, it is likely that ficolins deficiency would promote the increase of mortality. Thus, high rates of infant deaths in indigenous populations of the Arctic territories of Russia can, hypothetically, be associated with the immune system defects caused by the variants in ficolin genes. So far, no studies exploring this hypothesis were carried out, though such the studies would be able to determine the prevalence of inherited ficolin deficiencies and provide the ground for the development of prophylactic programmes for the timely identification and clinical examination of children with congenital ficolin defects, thus allowing to reduce the rates of infant deaths among aboriginal populations of Siberia.

The current study aimed to explore the prevalence of the genotypes of the *FCN2* gene for two single nucleotide polymorphisms, rs17549193 and rs7851696, known to be associated with severe course of bacterial infectious diseases, in ethnically different newborns of the aboriginal populations of Taymyr-Dolgan-Nenets Region of Krasnoyarskiy Kray of Russia (East Siberia).

Material and Methods

The study was approved by the Ethical Committee of the Scientific Research Institute of Medical Problems of the North (# 9 of 8.09.2014). Signed informed consent was obtained from parents of all participated children.

A total of 586 specimens of dried blood spots for the newborns from Taymyr-Dolgan-Nenets region of Krasnoyarskiy Kray were obtained from the Krasnoyarsk Regional Consulting-Diagnostic Centre for Medical Genetics to study the prevalence of single nucleotide polymorphisms of *FCN2* gene.

The newborns were split into four groups to study ethnic specificity of the *FCN2* polymorphisms (Table 1): 1, the Arctic region of mother's settlement, from villages with predominantly Nenets population (Nenets comprise 85% of the population); 2, the Arctic region of mother's settlement, from villages with predominantly Dolgan-Nganasan population (Dolgan-Nganasans comprise 91% of the population); 3, the Arctic region of mother's settlement, from villages with mixed populations with various combination of indigenous and alien residents; as a control group, 203 newborns from the city of Krasnoyarsk were recruited who had European ancestry (Russians) established via self-reports of their mothers.

Blood Sample Collection and Genotyping

DNA was extracted using DIAtom^{TM} DNA Prep kits (Centre for Molecular Genetics, Russia). Genotyping was carried out using restriction fragment lengths polymorphism (RFLP) approach. Two polymorphisms were studied, rs17549193 (+6359C>T; p.T236M) and rs7851696 (+6424G>T; p.A258S), both located in exon 8 of the gene. The relevant genomic fragment of 237 bp was amplified using the pair of oligonucleotide primers: forward 5'-CTGCCTGTAACGATGCTCAC-3' and reverse 5'-ATCCTTTCCCCGACTTCCAG-3' (annealing temperature 60˚C). Restriction endonucleases *HpySE526 I* (rs17549193) and *Mro X I* (rs7851696) for hydrolysis of the fragment followed by the electrophoresis in agarose gel with ethidium bromide to visualise the results. For rs17549193, *HpySE526 I* endonuclease produces a single fragment of 237 bp for the T allele and two fragments of 189 and 48 bp for the C allele. For rs7851696, *Mro X I* endonuclease produces a single fragment of 237 bp for the G allele and two fragments of 127 and 110 bp for the T allele.

Statistical Analysis

The compliance of the genotype frequencies with Hardy-Weinberg equilibrium was tested using either χ^2 or Fisher's exact tests. The comparison of the allele prevalence between the groups was carried out by χ^2 test using Gen Expert on-line calculator (http://gen-exp.ru/calculator_or.php). Odd's ratio (OR) and its 95% confidence intervals (CI) were calculated to estimate the strength of the association.

Results and Discussion

The analysis of the prevalence of the genotypes of the *FCN2* gene revealed the decreased frequency of the heterozygote genotype for the rs17549193 polymorphism in the newborns of the aboriginal Arctic populations of East Siberia as compared to the alien population (Table 2).

In Nenets, a TT genotype and T allele of the rs17549193 polymorphism were the rarest as compared to other populations (OR=0.06, CI=0.42-1.07, p=0.09 vs Dolgans-Nganasans and OR=0.38, CI=0.26-0.56, p<0.001 vs Russians) (Table 3). No statistically significant differences between the studied populations were established for the prevalence of genotypes and alleles for the rs7851696 polymorphism. However, it is notable that there is almost two-fold decrease of the prevalence of the T allele in Dolgans-Nganasans population as compared with Nenets and Russians from the city of Krasnoyarsk. Possibly, such the low prevalence of the beneficial genotype characteristic for the Nganasans can be confirmed in future in bigger samples.

Earlier, the minor allele at of the rs17549193 (+6359C>T) variant was found to be associated with a remarkable decrease of the binding capacity of L-ficolin with carbohydrate components of bacterial cell walls, while the minor allele of the rs7851696 (+6424G>T) was associated with the increased binding capacity (Hummelshoj et al. 2005). Also, it was shown that, in healthy Dutch donors, the plasma levels of L-ficolin decreased progressively depending on the number of the mutant allele of the rs7851696 causing amino acid substitutions in a gene–dose dependent manner. This suggests that the variant allele is linked to the high tissue activity of L-ficolin and, simultaneously, to its low concentration in plasma. No statistically significant association was seen between the L-ficolin serum concentration and the rs17549193 polymorphism in this Dutch cohort (Munthe-Fog et al. 2007). At the same time, some studies showed that high L-ficolin levels were associated with variant allele of rs17549193 (Cedzynski et al., 2007). Simultaneously, it was shown that this substitution is associated with the increased risk of visceral leishmaniasis and the increased levels of plasma L-ficolin (Mishra et al. 2015). This may suggest that the high plasma levels of L-ficolin are due to its decreased ability to bind the parasite (low avidity) thus resulting in its decreased accumulation in the site of inflammation.

Thus, the results obtained in the current study, suggest that the Arctic populations of East Siberia are characterised by specificity of genetic make-up responsible for the activity of L-ficolin. In the aboriginal populations of both Nenets and Dolgans-Nganasans, we found the decreased prevalence of the genotype for the rs7851696 polymorphism associated with low L-ficolin carbohydrates binding capacity, as compared to Russian population. Newborns in mixed arctic populations were characterized by the intermediate prevalence of the rs7851696 rare allele genotype. Because of the putative biological importance of bacterial carbohydrates binding capacity in lectins function, we suppose that Arctic populations are characterized by genetic predisposition to the higher level of L-

ficolin functional activity, as compared to Russian population. Additional studies are needed to establish the clinical significance of particular genotypes.

In the context of the current study, we think it is important to discuss the genetic differences between the Nenets and Dolgans-Nganasans with respect to the frequencies of the genotypes of the *FCN2* gene. The results of the study showed that the Nenets population exhibits several important features as compared with the Dolgans-Nganassans: lower prevalence of the allele T for the rs17549193 polymorphism and higher prevalence of the allele T for the rs7851696 polymorphism. We believe that this genotype is a genetic marker of high functional capacity of L-ficolin in Nenets population. In our earlier study, for the first time in Russia, we analyzed the prevalence of a single nucleotide polymorphism rs80356779 (c.1436C→T) in the gene coding carnitine palmitoyltransferase type 1A (CPT1A) (so called "Arctic variant") in newborns of the aboriginal populations of Taymyr Dolgano-Nganasan Region of Krasnoyarskiy Kray and the city of Krasnoyarsk (Tereshchenko and Smolnikova 2016). We showed that as little as 7% of the Dolgan-Nganasan population carried the rare T-allele of the *CPT1A* gene, while no carriers of the «Arctic variant» were present in the Nenets population. Taking into account that the carriers of the rare T-allele of the *CPT1A* gene are susceptible to more severe course of infectious diseases (Gessner et al. 2010) and given the results of the current study of the *FCN2* genotypes, we suggest that the Dolgan-Nganasan population exhibit the increased liability to severe and unfavourable course of early age infections, thus directly affecting the epidemiological figures of infant mortality in this ethnic group.

Conclusion

The results of the current and our previous study suggest that the Nenets population has the highest level of non-specific anti-infectious defence as compared with other populations of East Siberia. This can be taken into account for more effective planning of the use of the healthcare resources in the North. Also, additional analysis of infectious disease morbidity in the studied populations will allow revealing phenotypic characteristics of the Nenets population associated with the increased functional capacity of L-ficolin as one of the important agent of the first line defence agent infection. The analyses of the relationships between clinical and genetic traits are crucially important for understanding of the real physiological role of L-ficolin, and the established genetic features in ethnically isolated Nenets populations provide a unique opportunity for such the study.

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Fig. 1

A schematic representation of the human FCN2 gene. Exon 1 encodes signal peptide and start of N-terminal region. Exon 2-3 encodes remainder of N-terminal region and collagen-like region. Exon 4 encodes linker region. Exon 5-8 encodes fibrinogen-like domain. In the current study, two polymorphisms in exon 8 (+6359 and +6424) were analyzed. Reproduced from Kilpatrick et al., 2012, with modifications (Kilpatrick and Chalmers 2012)

Table 1 The prevalence of the studied newborns according to the region of mother's settlement

Group	The Arctic region of mother's settlement	N	Relative frequency (%)	Ethnic composition of the settlement (total / aboriginal)
Nenets	Nosok	106	27.7	1692 / 1370
	Tukhard	20	5.2	922 / 858
Dolgans-Nganasans	Sindassko	35	9.1	523 / 496
	Katarik	28	7.3	362 / 334
	Novaya	25	6.5	313 / 247
	Levinskie Peski	2	0.5	112 / 112
Mixed aboriginal population	Novoribnaya	25	6.5	635 / 556
	Ust-Avam	24	6.3	513 / 300
	Volochanka	18	4.7	530 / 300
	Kheta	17	4.4	368 / 368
	Zhdanikha	15	3.9	205 / 205
	Popigai	15	3.9	334 / 334
	Vorontsovo	12	3.1	310 / 246
	Khantaiskoe Ozero	11	2.9	354 / 224
	Kresti	10	2.6	274 / 274
	Khatanga	10	2.6	5416 / 3908
	Ust-Port	4	1.1	331 / 184
	Baikalovsk	3	0.8	131 / 116
	Karaul	2	0.5	801 / 760
	Ust-Eniseisk	1	0.4	No data available

Table 2 The prevalence of the *FCN2* genotypes in the newborns from different ethnic populations of Taymyr-Dolgan-Nenets region of Krasnoyarskiy Kray and the city of Krasnoyarsk

***FCN2* genotype**		**Nenets (n=126)**	**Dolgans-Nganasans (n=90)**	**Mixed Arctic populations (n=167)**	**Russians (n=203)**
		1	2	3	4
rs17549193	CC	82 (65.1%)	51 (56.6%)	95 (56.9%)	72 (35.4%)
(+6359C>T)	CT	42 (33.3%)	33 (36.7%)	64 (38.3%)	112 (55.2%)
(p.T236M)	TT	2 (1.6%)	6 (6.7%)	8 (4.8%)	19 (9.4%)
	T*	0.37	0.50	0.48	0.74
rs7851696	GG	108 (85.7%)	83 (92.2%)	148 (88.6%)	174 (85.7%)
(+6424G>T)	GT	17 (13.5%)	7 (7.8%)	19 (11.4%)	27 (13.3%)
(p.A258S)	TT	1 (0.8%)	0 (0%)	0 (0%)	2 (1.0%)
	T*	0.15	0.08	0.11	0.15

* – the variant allele in the studied populations

Table 3 Odd's ratios (95% CI) and p-values for comparisons of *FCN2* allele prevalence in the newborns from different ethnic groups of Taymyr-Dolgan-Nenets region of Krasnoyarskiy Kray and the city of Krasnoyarsk

Populations	**Dolgans-Nganasans**	**Mixed Arctic populations**	**Russians**
		rs17549193	
Nenets	p = 0.09 OR = 0.06 (0.42-1.07)	p = 0.1 OR = 0.71 (0.47-1.06)	p < 0.001 OR = 0.38 (0.26-0.56)
Dolgans-Nganasans	–	p = 0.79 OR = 1.06 (0.69-1.61)	p = 0.005 OR = 0.57 (0.38-0.84)
Mixed Arctic populations	–	–	p < 0.001 OR = 0.54 (0.39-0.74)
		rs7851696	
Nenets	p = 0.12 OR = 2.02 (0.83-4.90)	p = 0.37 OR = 1.35 (0.7-2.61)	p = 0.96 OR = 0.99 (0.54-1.79)
Dolgans-Nganasans	–	p = 0.37 OR = 0.67 (0.28-1.63)	p = 0.09 OR = 0.49 (0.21-1.13)
Mixed Arctic populations	–	–	p = 0.29 OR = 0.73 (0.4-1.32)